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**Original Article**

# A Nosocomial Outbreak of Febrile Bloodstream Infection Caused by Heparinized-Saline Contaminated with *Serratia marcescens*, Tokyo, 2002

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**SUMMARY**: In January 2002, 12 patients with *Serratia marcescens* bloodstream infection (BSI) were identified in a hospital in Tokyo, Japan. We conducted an epidemiological investigation of this outbreak. We undertook a medical-records review and employee interviews, and performed a case-control study to determine risk factors for *S. marcescens* BSI. An observational study of the hospital's procedures and an environmental microbiologic sampling were performed. We identified 12 suspected and 12 confirmed patients with *S. marcescens* BSI, including 7 who died. A case-control study showed that vascular access devices (odds ratio [OR] = 30.46; 95% confidence interval [CI] = 3.5 - 685.6) and the use of heparin-locks, between December 26 and January 15, (OR = 25.7; 95% CI = 2.3 - 680.4) were significant risk factors for *S. marcescens* BSI. The observational study revealed multiple lapses in infection control, including use of multi-dose vials of heparin. The outbreak strain was isolated from a hand-towel in the nurse station. The use of multi-dose vials of heparinized-saline during a particularly busy period was associated with BSI risk. The results underscore the risks inherent in infection-control lapses and the use of multi-dose vials.

## INTRODUCTION

*Serratia marcescens* was once considered nonpathogenic in humans, but now is known to be a significant cause of nosocomial infections (1,2).

In January 2002, a public health center in Tokyo received reports of 12 febrile patients and a cluster of *S. marcescens* isolates at a small-scale neurosurgical hospital (hospital A). The Field Epidemiology Training Program-Japan was invited to support the investigation of this outbreak.

## METHODS

**Case definitions**: A suspected case was defined as a person admitted to hospital A for at least 1 day with a fever greater than 38.5°C and without any other focal signs of infection, including respiratory and urinary tract infection, between December 20, 2001 and January 15, 2002 (the study period). A confirmed case had in addition a positive blood culture for *S. marcescens*, with the same antibiogram and PFGE pattern as the index case.

**Case-control study**: We reviewed medical and nursing records of patients admitted during the study period and interviewed employees. Data were collected, including demographic information, admission diagnosis, room number, activity of daily life (ADL) level, date of onset of fever, symptoms, clinical outcomes, medications, type of vascular access, and bacteria isolated from clinical cultures. We investigated procedures in the hospital and any problems identified before this incident.

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**Observational study**: We observed hospital A practices, including environmental hygiene, and medical procedures, e.g., preparation of injectable drugs, care of vascular access sites, dressing changes, and wound care.

**Laboratory studies**: A total of 158 samples were collected for bacterial culture from environmental surfaces, including sinks, floors, walls around beds, and medical equipment, from water in medical equipment, and from the hands of employees, vascular access lines and stored injectables. Clinical laboratory data were reviewed for isolates of *S. marcescens* at hospital A over the previous 12 months. Confirmatory culture and endotoxin assays were performed on some of the patient serum samples.

**Statistical analysis**: Data entry and univariate analysis were performed using Epi Info (version 6; Centers for Disease Control and Prevention, Atlanta, Ga, USA).

## RESULTS

**Descriptive epidemiology**: Hospital A had 33 beds on two floors, with a surgical suite and imaging equipment (computed tomography [CT] and magnetic resonance [MR]) in the basement.

We identified 12 suspected and 12 confirmed cases. One suspected and six confirmed cases died of a sepsis-like syndrome complicated by disseminated intravascular coagulation and multiple-organ failure. Ages ranged from 20 to 96 years old (median, 70 years old) and the male:female ratio was 1:1.67. All case-patients but one with subarachnoid hemorrhage (initial fatal case) had been stable until sudden onset of fever. In some cases, death occurred within a few days. Most *S. marcescens* isolates were from blood, though two were from urine. All case-patients had some type of vascular access.

The epidemic curve (Fig. 1) shows that cases occurred

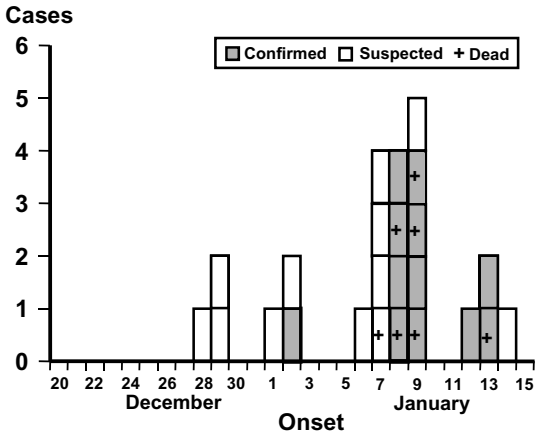


Fig. 1. Date of onset of *S. marcescens* bloodstream infection cases in the hospital A, Tokyo, 2001 - 2002.

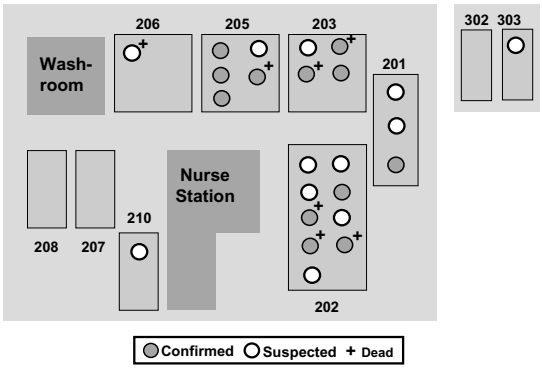


Fig. 2. Allocation of *S. marcescens* bloodstream infection cases in the ward in the hospital A, Tokyo, 2001 - 2002.

between December 28, 2001, and January 14, 2002. Cases formed four clusters separated by 4 - 5 days.

Figure 2 shows the distribution of case-patients in the hospital. No localized distribution was observed. Medical records also showed that no specific employees were responsible for cases 1 week before their onset.

**Observational study**: Several problems were identified at hospital A. Drugs for injection were prepared at a nurse station adjacent to a sink used for both hand-washing and for cleaning soiled medical equipment. We also found problems in medical waste disposal. Hand-washing was not performed prior to patient contact and chlorinated water was used instead of alcohol for disinfection. Heparin-locks, the filling of intravenous access (IV) sites with a heparin solution to prevent clotting, were used frequently, especially for patients receiving intermittent infusion or whose infusion was disconnected during CT or MR imaging. Heparinized-saline for use in heparin-locks was made by adding 5 mL of heparin to a bottle of 500 mL saline. Several opened heparin vials, from which nursing staff withdrew 1 or 2 mL to add to saline, were kept in a refrigerator. The diluted solution was prepared at a nurse station and kept at room temperature on the table where it was prepared. This supply of heparinized-saline was commonly used for 3 - 5 days until it was gone.

**Case-control study**: Twenty-one case-patients were compared with 29 control-patients selected randomly from among inpatients during the study period. Control-patients were 19 to 92 years old (median, 70 years old); 48% were male. We excluded three cases from the study. In two of these cases, *S. marcescens* was isolated only from urine. The third case had a urinary tract infection caused by *Proteus mirabilis*.

Table 1. Results of case-control study

| | | | | Odds ratio | 95% CI |
|---|---|---|---|---|---|
| | | + | – | | |
| Vascular access | Case | 21 | 0 | | |
| | Control | 12 | 17 | 30.46 | 3.48 - 685.63 |
| | | Cont. | Int. | | |
| Infusion | Case | 17 | 4 | | |
| | Control | 0 | 12 | 46.80 | 4.23 - 1229.07 |
| | | + | – | | |
| Heparin-lock | Case | 21 | 0 | | |
| | Control | 12 | 0 | 1.69 | 0 - 69.25 |
| | | + | – | | |
| Before Dec. 25 | Case | 7 | 2 | | |
| | Control | 9 | 1 | 1.75 | 0 - 70.17 |
| | | + | – | | |
| Others* | Case | 21 | 0 | | |
| | Control | 5 | 7 | 25.67 | 2.25 - 680.43 |
| | | + | – | | |
| Airway suction | Case | 10 | 11 | | |
| | Control | 1 | 28 | 25.45 | 2.70 – 597.96 |
| Nebulizer | Case | 9 | 12 | | |
| | Control | 1 | 28 | 21.00 | 2.22 – 444.17 |
| Intubation | Case | 9 | 12 | | |
| | Control | 0 | 29 | 23.08 | 2.52 – 533.62 |
| Urinary catheter | Case | 16 | 5 | | |
| | Control | 9 | 20 | 7.11 | 1.70 – 31.77 |

CI: Confidence Interval; Cont.: Continuous infusion; Int.: Intermittent infusion.
*: Those who received heparin lock before and after Dec. 25 and only after 26.

Table 1 shows the results of the case-control study. Having vascular access was a significant risk factor (odds ratio [OR] = 30.46; 95% confidence interval [CI] = 3.48 - 685.63) for *S. marcescens* BSI. Compared with intermittent infusion, continuous infusion was associated with a higher risk of being a case-patient (OR = 46.80, 95% CI = 4.23 - 1229.07). Heparin-lock use, present in 33 patients with vascular access (21 cases, 12 controls), was not associated with BSI risk (OR = 1.69, 95% CI = 0-69.25). However, we noticed the controls before December 25 were admitted with mild injuries and were discharged soon thereafter. The nurses informed us that they became busier after December 26 due to the New Year season. We stratified the data by this date, and determined that those for whom heparin-locks were used after December 25 had a significantly higher risk of infection than those for whom heparin-locks were used on or before December 25 (OR = 25.67, 95% CI = 2.25 - 680.43).

We could estimate the time of exposure in 19 out of 21 case-patients. We plotted the interval between exposure and onset, dividing the cases according to outcome (Fig. 3). The interval ranged from 0 to 7 days (median, 1 day) and fatal cases had shorter intervals compared with improved cases.

Other risk factors, including respiratory suctioning, nebulizer use, and intubation, are shown in Table 1.

**Laboratory studies**: *S. marcescens* was isolated from three environmental samples. One isolate was from medical adhesive tape and one from a three-way valve that was part of a patient IV line. Both had PFGE patterns that were

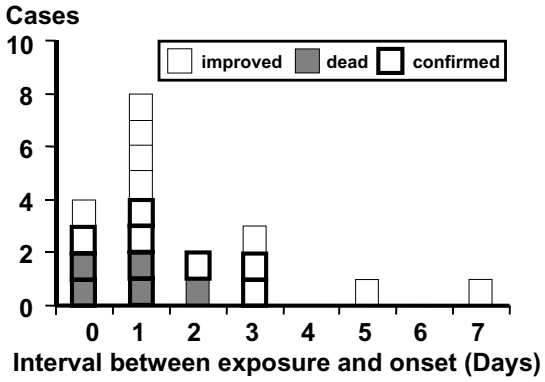


Fig. 3. Interval between probable exposure (heparin-lock) and onset of *S. marcescens* bloodstream infection cases in the ward in the hospital A, Tokyo, 2001 - 2002.

different from the outbreak strain. The third isolate was from a towel found at the sink in the nurse station. This towel was used to dry medical equipment after washing. The isolate from the towel was indistinguishable from the outbreak strain. *S. marcescens* was not isolated from any of the opened heparin vials. The heparinized-saline in use during this outbreak had already been used and was no longer available for culture. In the past 12 months, there were four *S. marcescens* isolates from urine and sputum of patients with poor ADL.

Serum endotoxin was determined in serum from 19 case-patients (12 confirmed and 7 suspected). The sera from one suspected and six confirmed cases were positive. Five of these came from patients who died. Two specimens from fatal cases showed very high concentrations (>1,512,270 pg/mL and >19,840 pg/mL, respectively).

## DISCUSSION

This outbreak involved 24 patients and contributed to at least 7 deaths. Patients with vascular access who had heparin-locks during a period of increased patient census were found to be at significant risk of infection. Although heparin solution samples were not available for culture, the finding of the outbreak strain in the environment where the solution was prepared seems to support our hypothesis that contaminated heparinized-saline was a partial cause of this outbreak.

*S. marcescens* is a pathogen known to cause nosocomial infections (1,2). Outbreaks have been reported in association with a variety of sources, including ECG electrodes (3), bronchoscope (4), disinfectants such as benzalkonium-chloride (5) and hexitidine (6), and even hand-lotions (7). It also has caused sepsis among injecting heroin users (1). However, reports of outbreaks caused by contamination of injectable drugs in clinical settings are rare. Only five outbreaks have been reported, one involving an anesthetic agent, propofol (8), three due to heparin (9-11) and one related to erythropoetin alpha (12); the two lattermost outbreaks (11,12) involved the species *S. liquefaciens* rather than *S. marcescens*. Therefore, to the best of our knowledge, there have been only three *Serratia* outbreaks caused by contaminated heparin worldwide (9-11), and two of them were caused by *S. marcescens* (9,10). We believe our report is the first account of *S. marcescens* bacteremia caused by injection of contaminated medication in Japan, where *S. marcescens* nosocomial outbreaks are less commonly reported than in Western countries.

In most nosocomial outbreaks, *S. marcescens* first colonizes the respiratory (13) or urinary (14) tract. Investigations have demonstrated associations between paticular medical procedures and risk of *Serratia* infection. In this outbreak, four procedures – airway suction, nebulizer use, intubation, and urinary catheterization – were significant risk factors in univariate analysis. *S. marcescens* is usually known to cause infections in debilitated and hospitalized persons (1,3). Consequently, risks associated with some procedures might reflect the inherent risk for the patient population undergoing them.

In regard to the incubation period in this outbreak, we speculate the following. First, the reason that all five fatal cases in Fig. 3 had short incubation periods was likely because they received a relatively high dose of *S. marcescens*. This was supported by the high endotoxin levels in these patients. On the other hand, two cases had long incubation periods of 5 and 7 days. These two patients might have had fevers from other causes. However, *S. marcescens* is known to adhere easily to the walls of plastic tubes and bags (15,16). If the pathogen adhered to one of these walls and proliferated there for several days before invading the bloodstream, this would explain the long incubation period.

In this outbreak, the epidemic curve showed multiple peaks every 4 or 5 days. Nurses prepared heparinized-saline by drawing a small amount of heparin from one of several vials every 4 or 5 days. The number of pathogens may have been too small to cause infection just after the preparation, but may have increased sufficiently to cause septicemia when the bottle was left at room temperature for several days.

The affected ward was closed by the order of the sanitary division of the Tokyo Metropolitan Office. As noted by Ogtrop et al. (17) based on experience in a neonatal care unit setting, control of nosocomial outbreaks caused by this pathogen can be difficult and requires improvement in the performance of medical procedures. They concluded that the best control could be achieved by closing the hospital. This might partly support the decisions made by the Tokyo sanitary division. However, we caution that similar outbreaks may occur, since the heparin vial is designed to be punctured multiple times, and heparin-lock is a common procedure. We recommend that pharmacies and medical device producers develop single-use heparin solutions and vascular access devices that require no heparin-lock.

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